



3 Mar 2017

**Response to EPA Questions (received 1 March 2017) regarding tTAV and DsRed2 protein expression/stability in OX513A larvae/adults**

**1.) Are there quantitative data on the amounts of tTAV and dsRed proteins in larval or adult mosquitoes?**

We have previously shown, using Western blot analyses, that tTAV and DsRed2 proteins are not present at detectable levels (LOD for recombinant Ttav is 0.8 ng and for DsRed2 it is 2.5-5.0 ng) in the saliva of OX513A adult female mosquitoes, which is the only likely route of exposure to these proteins in humans (see p.96 of the FDA EA, 5 Aug 2016, for a summary of these findings, and Appendix K of the FDA EA for details: [ [HYPERLINK "https://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm490246.htm"](https://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm490246.htm) ]). We have not measured the quantity of tTAV and DsRed2 proteins in larval or adult mosquitoes.

**2.) What is the relative molecular mass for tTAV protein?**

**The calculated molecular mass is 37 kDa.** The protein sequence and standard parameters report (calculated using the Expasy Protparam tool) is given below:

OX513A tTAV Protein Parameters  
<http://web.expasy.org/protparam/>

10	20	30	40	50	60
MGSRLDKSKV	INSALELLNE	VGIEGLTTRK	LAQKLGVEQP	TLYWHVKNKR	ALLDALAIEM
70	80	90	100	110	120
LDRHHTHFCP	LEGESWQDFL	RNNAKSFRCA	KILLSHRDGAKV	H LGTRPTEKQ	YETLENQLAF
130	140	150	160	170	180
LCQQGFSLEN	ALYALSAVGH	FTLGCVLEDQ	EHQVAKEERE	TPTTDSMPPL	LRQAIELFDH
190	200	210	220	230	240
QGAEP AFLFG	LELIICGLEK	QLKCESGSGP	AYS RARTKNN	YGSTIEGLLD	LPDDDAPEEA
250	260	270	280	290	300
GLAAPRLSFL	PAGHTRRLST	APPTDVSLGD	ELHLDGEDVA	MAHADALDDF	DLDMLGDGDS
310	320	330			
PGPGFTP HD S	APYGA LD MAD	FEFEQMFTDA	LGIDEYGG		

Number of amino acids: 338

Molecular weight: 37121.56 Da

**3.) How stable is tTAV to changes in ionic strength, pH or other physical conditions (how stable is relative to other proteins)?**

There are many literature reports of the use of the tTA system and its variants in a wide range of gene regulation studies (see [ [HYPERLINK "http://www.tetsystems.com/science-technology/highlighted-publications/"](http://www.tetsystems.com/science-technology/highlighted-publications/) ]). Tetracycline induced gene expression is frequently heterogenous and this has been attributed to the instability of tTA , due to cryptic splicing sites and prokaryotic codons (Qi et al, 2004). However, despite this wide variety of literature on the use of tTA and its variants in gene regulation we can find no published papers on its stability

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in physical conditions. We do not have experimental data regarding the relative stability of tTAV in different pH/ionic strength/temperature conditions.

(Qi et al (2004) Gene 327(1) pg61-73)

4.) Is tTAV known to be susceptible to any specific proteases?

## Protease digest prediction tools [ HYPERLINK ]

"file:///C:/Users/camilla.beech/AppData/Local/Microsoft/Windows/INetCache/Content.Outlook/DURQCK2Y/(http://web.expasy.org/peptide\_cutter/)" ] identify multiple digestion sites for Trypsin, Chymotrypsin and Pepsin (each cut site is C-terminal to the highlighted amino acid), indicating that this protein is likely to be rapidly and efficiently digested by proteases:

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**OX513A tTAV Protease Digest Analysis: Chymotrypsin High Specificity**  
[http://web.expasy.org/peptide\\_cutter/](http://web.expasy.org/peptide_cutter/)

	Ch_hi Ch_hi 	
1	MGSRLDKSKVINSALLELLNEVGIEGLTTRKLAQKLGVEQPTLYWHVKNKRALLDALAIEM	60
	Ch_hi      Ch_hi      Ch_hi      Ch_hi      Ch_hi      Ch_hi	
61	LDRHHHTHFCPLEGESWQDFLRNNNAKSFRCALLSHRDGAKVHLGTRPTEKQYETLENQLAF	120
	Ch_hi      Ch_hi      Ch_hi      Ch_hi	
121	LCQQGFSLENALYALSAGVHFTLGCVLEDQEHQVAKEERETPTTDSMPPLLQAIELFDH	180
	Ch_hi      Ch_hi      Ch_hi      Ch_hi	
181	QGAEPAPFLGLELEIICGLEKQLKCESGSGPAYSARTKNNGSTIEGLLDLPDDDAPEEA	240
	Ch_hi      Ch_hi	
241	GLAAPRLSFLPAGHTRRLSTAPPTDVSLGDELHLDGEDVAMAHADALDDFDLDMLGDGDS	300
	Ch_hi      Ch_hi      Ch_hi      Ch_hi      Ch_hi	
301	PGPGFTP HD S A P Y G A L D M A D F E F E Q M F T D A L G I D E Y G G	338

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## **OX513A tTAV Protease Digest Analysis: Pepsin (pH>2.0)**

[http://web.expasy.org/peptide\\_cutter/](http://web.expasy.org/peptide_cutter/)

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